Nymphaea nouchali var. *versicolor* 'Bua Phuean': Seed Morphology and Germination In Vitro

Kitti Bodhipadma[1*], Sompoch Noichinda[1], Phetcharat Wachirabongkoth[1], Ekawut Pukpoomin[1], Luepol Punnakanta[2] and Koravisd Nathalang[3]

[1]*Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangsue, Bangkok 10800, Thailand*

[2]*Faculty of Environment and Resource Studies, Mahidol University, Salaya, Nakhon Pathom 73170, Thailand*

[3]*Department of Biology, Faculty of Science, Mahidol University, Rama VI Rd., Rajathevi District, Bangkok 10400, Thailand*

Abstract

Nymphaea nouchali var. *versicolor* 'Bua Phuean' is one of Thai native species of day-blooming waterlily. Since the expansion of community from metropolitan area and the appreciation on exotic species of *Nymphaea*, number of this plant in environment is noticeably decreased. One of the efficient methods to conserve and increase the amount of this plant is seed germination. Thus, seed morphology and developmental stages of 'Bua Phuean' were investigated before germinating in vitro. The results revealed that 'Bua Phuean' seeds could be categorized into 5 stages and each stage also had dissimilar morphology. After the stages 3 to 5 seeds were germinated under aseptic condition, seed in stage 5 showed the highest germination rate at 82.22 %. During seed germination, coleoptile emerged from seed coat before root became visible.

Key words: aseptic condition/ germination rate/ *Nymphaea nouchali*/ seed stage/ seedling development

1. Introduction

Nymphaea is the largest genus in the family Nymphaeaceae with about 40 species. The genus is spread out all continents except Antarctica (Orban and Bouharmont, 1998). Many species of the genus in Thailand are grown as ornamental plants. However, there is only one native species of day-blooming waterlilies, *N. nouchali* Burm. f. (*N. stellata*). In Thailand, two varieties are recgnized which are *versicolor* and *cyanea*. For *N. nouchali* var. *versicolor*, it has two separate forms: 'Bua Phan' and 'Bua Phuean' while *N. nouchali* var. *cyanea* is known as 'Bua Khap'. 'Bua Phuean' typically has smaller flower than 'Bua Phan'. The flower has white petal with pale bluish purple tip and does not change color after blooming (Chomchalow and Chansilpa, 2007) (Figure 1).

Due to the extension of population from the metropolis that invaded growing area and the admiration on exotic species of *Nymphaea*, the Thai native species of day-blooming waterlily are currently threatening, especially 'Bua Phuean'. Despite the fact that this plant variety could reproduce asexually from its tuberous rhizome, propagation through seed germination would successfully increase the number and reduce the loss of plants in nature. Seed germination of *Nymphaea* had been studied in many species such as, fragrant waterlily (*N. odorata*) (Else and Riemer, 1984), *N. lotus* (Mohammed and Awodoyin, 2008) and white water lily (*N. alba*) (Estrelles et al., 2004; Sumlu et al., 2010). Nevertheless, germination of 'Bua Phuean' seed, particularly in vitro, has never been reported. Therefore, the purposes of this research were to examine 'Bua Phuean' seed morphology, classify the seed stages, and investigate the development and rate of seed germination in vitro.

*Corresponding author
Email: kbm@kmutnb.ac.th

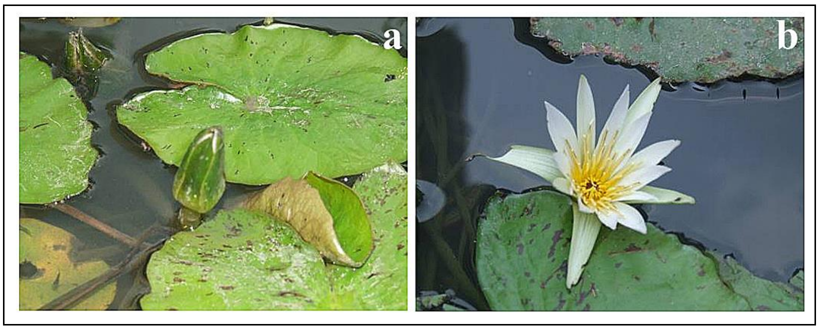

Figure 1 Flower of 'Bua Phuean' before (left) and after (right) blooming

2. Methodology

Fresh fruits of *N. nouchali* var. *versicolor* 'Bua Phuean' at various stages were gathered from natural pond in Pathum Thani province, Thailand. After fruits had been cut open, seeds were taken from the fruits and investigated under a stereomicroscope (EMZ-TR, Meiji Techno Co., Ltd.) to classify seed developmental stages from their morphology. Then, some stages of seed (stage 3 to 5) were used for in vitro germination.

Seeds of each selected stage were separately rinsed with clean running tap water for 10 min and washed in distilled water. Seeds were immersed for 15 min in 5% (v/v) Clorox (a commercial bleach solution containing 5.25%, w/w, sodium hypochlorite as available chlorine) to which 2 drops of Tween-20 were added. The samples were rinsed 3 times with sterile distilled water each for 5 min before placing on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962). This medium was adjusted to pH 5.7, gelled with 0.8% (w/v) agar, and autoclaved at 121 °C and 15 psi for 20 min. Afterward, 15 ml of sterile distilled water was poured over the seeds on semi-solid medium. Cultures were then kept in a growth room under dark condition or 16 hr of illumination from cool white fluorescent lamps (44.57 $\mu mol/m^2/s$ light intensity) and 8 hr of darkness at 25±2 °C. Data were collected until the first foliage leaf of 'Bua Phuean' seedling had fully expanded.

3. Results and discussion

N. nouchali var. *versicolor* 'Bua Phuean' is an aquatic perennial herb. The fruit is berry-like and spongy. When various stages of 'Bua Phuean' fruits had been cut open, numerous seeds were found inside (Figure 2). With the naked eye, the different color of seeds could be seen in dissimilar stages of fruits. According to the diverse seed colors, 'Bua Phuean' fruits were classified into stage 1 to 5 which contained orange-red, red, light brown, brown and dark brown seeds, respectively (Figure 2).

After investigation under a stereomicroscope, these seeds were ellipsoid-globose and able to be arranged into 5 stages (Figures 3 and 4): stage 1: seed coat was orange-red and without pubescent longitudinal ridges pubescent absent, stage 2: seed coat was red with longitudinal rows of hairs, stage 3: seed coat was light brown, rows of longitudinal trichomes present and pulpy aril started developing from one end, stage 4: seed coat was brown, rows of longitudinal trichomes present and air-containing sack-like, pulpy aril covered, stage 5: seed coat was dark brown, rows of longitudinal trichomes present and thick air-containing sack-like, pulpy aril covered.

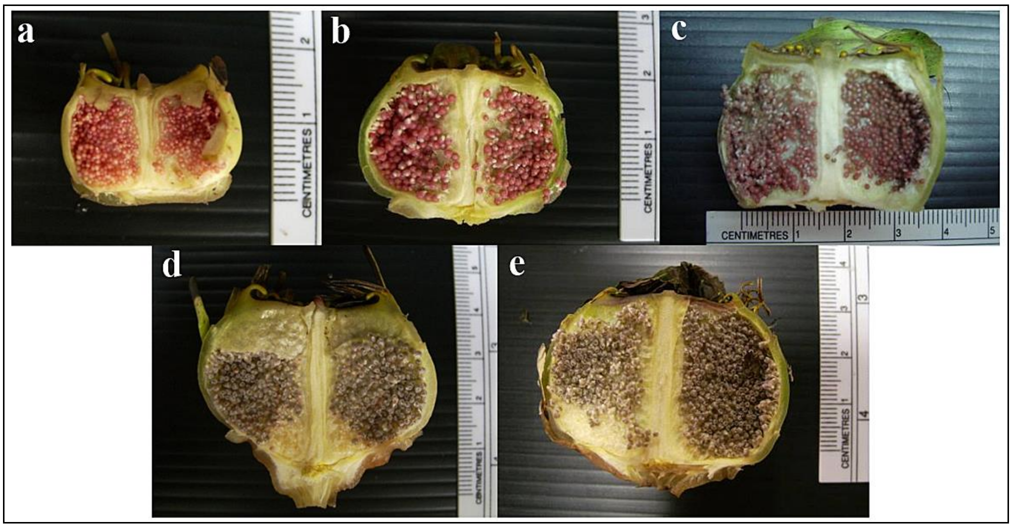

Figure 2 Fruits of 'Bua Phuean' at various stages (stage 1: a, stage 2: b, stage 3: c, stage 4: d and stage 5: e) after they were cut open

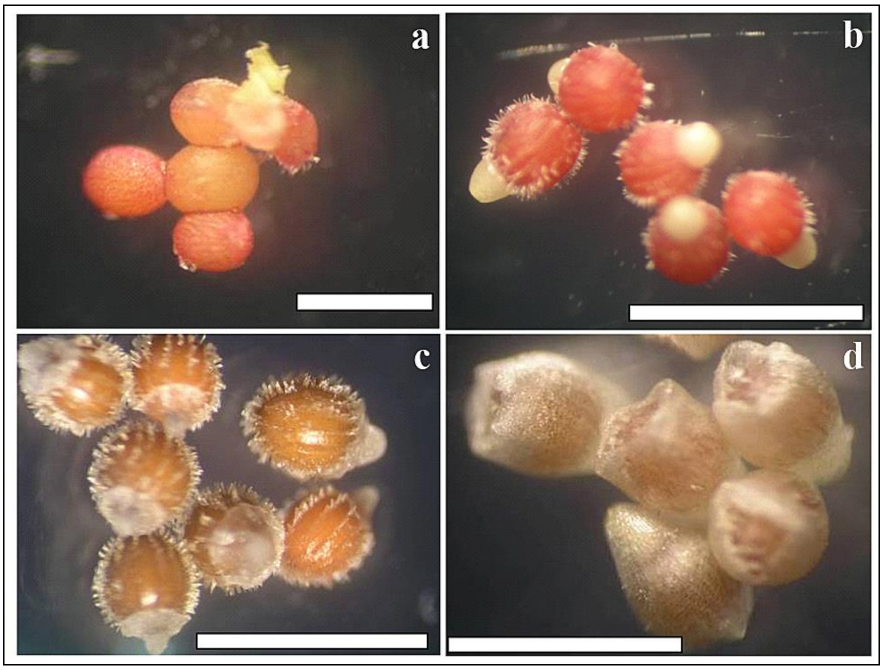

Figure 3 Seeds of 'Bua Phuean' at different stages: stage 1 (a), stage 2 (b), stage 3 (c) and stage 4 (d), scale bar = 1 mm

From our preliminary test on 'Bua Phuean' seed germination, stages 1 and 2 seeds did not germinate after sowing (data not shown). The reason of this might occur from seed maturity. Seeds from stages 1 and 2 may possibly contain young embryo that lacking of germinability. In contrast, stages 3 to 5 seeds could germinate well under aseptic condition. However, the capability of seed germination was found different in dissimilar stages of seeds (Figure 5).

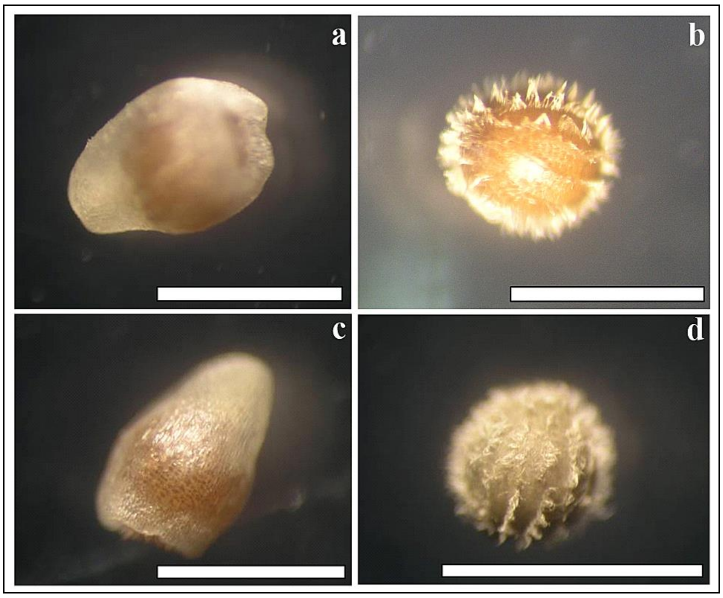

Figure 4 Seeds with (left) or without (right) air-containing sack-like, pulpy aril of 'Bua Phuean' at stage 4 (a and b) and 5 (c and d), scale bar = 1 mm

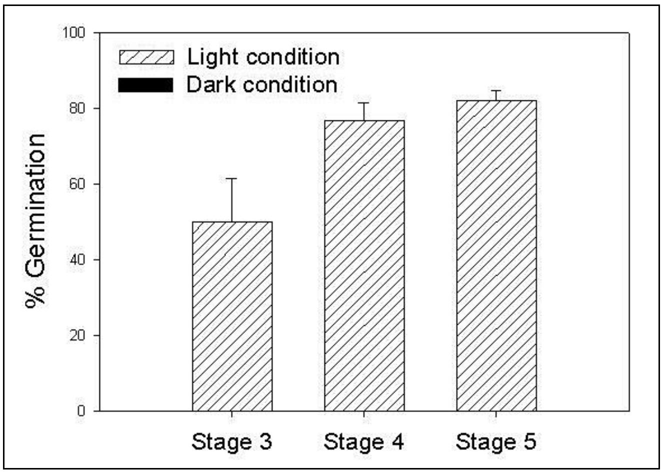

Figure 5 Germination percentage of 'Bua Phuean' seeds (stage 3 to 5) in vitro under light and dark conditions (values are means of 3 replicates±S.E.)

One of the unique characters of *Nymphaea* seeds is the whitish or translucent aril (Bonilla-Barbosa et al., 2000). This aril was found covering 50% longer than 'Bua Phuean' seed in stage 5 (Figure 4). At some point in seed surface sterilization, spongy aril was mangled. Thus, all surface-sterilized seeds that placing on the MS basal medium was uncovered with the aril. Our result indicated that stages 3, 4 and 5 seeds started germinating on week 8, 4 and 2, respectively, while the first foliage leaf of those stages completely expanded on week 10, 6 and 4, consecutively. The highest germination percentage was obtained from stage 5 seeds at 82.22 (Figure 5). These findings illustrated that mature seeds of 'Bua Phuean' had higher germinability than the younger ones. The older seeds evidently consumed less time for germination than the younger ones though stages 4 and 5 seeds appeared to have close number of germination percentage. Moreover, the results also suggested that light promoted germination in 'Bua Phuean' seeds as none of seeds from stage 3 to 5 were found germinating under dark condition (Figures 5 and 6).

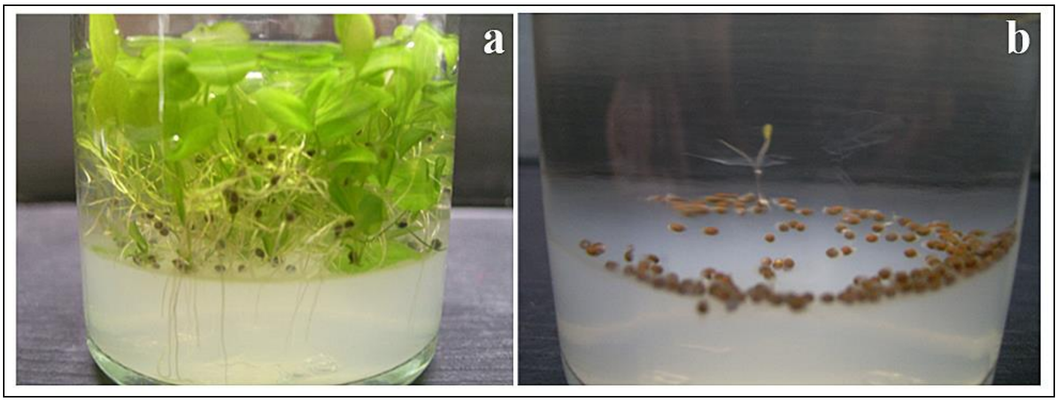

Figure 6 In vitro seed germination of 'Bua Phuean' under light (a) and dark (b) conditions

Seed germination of *Nymphaea* had been studied both aseptic and non-aseptic conditions. This occurrence was diverse among those species. The non-aseptic condition, fragrant waterlily seeds stored in dark germinated only 2% whereas seeds kept in the light gave around 30% germination (Else and Riemer, 1984). In white water lily, the immature seeds had lower germination percentage than mature ones and the best germination rate was about 80% (Estrelles et al., 2004). This percentage of germination was the same as in *N. lotus* (Mohammed and Awodoyin, 2008). In vitro condition, white water lily seeds cultured on agar solidified MS medium containing 1 mg/l BAP and 0.1 mg/l IAA provided the highest germination (60.20%) and there was no significant difference between light and dark germination (Sumlu et al., 2010). In contrast, light became the important factor in stimulating ex vitro seed germination of white water lily (Smits et al., 1990). These results exhibited that aseptic and non-aseptic conditions could have some effects on this phenomenon in white water lily but did not affect germinability of 'Bua Phuean' seeds in vitro.

Subsequent to seed germination, 'Bua Phuean' seedling development was observed under a stereomicroscope as well. The coleoptile (plumular sheath) protruded out of seed coat before the first foliage leaf emerged (Figure 7). Afterward, root became noticeable and the first foliage leaf began expanding. This process was unlike the growth of *N. lotus* var. *pubescens* seedling because, in this variety, a ring of long rhizoids formed around the base of hypocotyl following the emergence of coleoptile (Philomena and Shah, 1985). Nonetheless, this ring of long rhizoids was not found initiating in 'Bua Phuean' seedling. So far, figure of seedling development in *Nymphaea* from many publications was rare. Sometimes, it was illustrated by drawing (Philomena and Shah, 1985) but the present research demonstrated real pictures of 'Bua Phuean' seedling development at each stage for the first time (Figure 7).

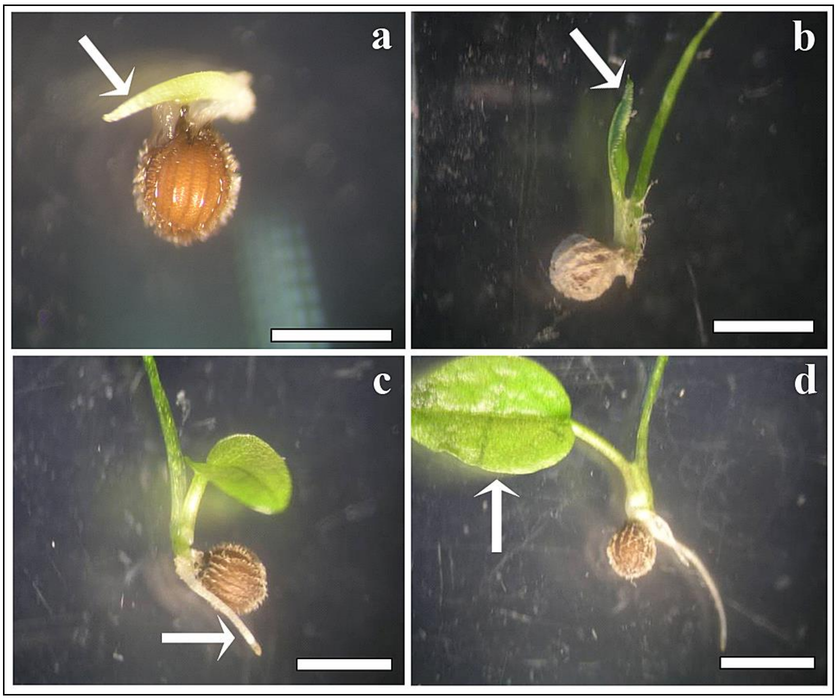

Figure 7 'Bua Phuean' seedling development under light condition:
(a) protruding coleoptile (arrow), (b) emergence of first foliage leaf (arrow),
(c) root elongation (arrow), (d) fully expanding first foliage leaf (arrow), scale bar = 1 mm

4. Conclusion

Natural mechanism is quite elaboration and complication. All living things evolutionarily use this mechanism to sustain their descendants. Propagation only on highly demanded species is inadequate to support global and environmental changes. Thus, conserving the endangered species should really take into consideration during technology development. This is in order to maintain the evolutionary and natural directions. Development of seed propagation technology, therefore, would be the valuable know-how which underlines the need of multiplication and upholds the evolutionary direction and natural breeding.

The number of *Nymphaea nouchali* var. *versicolor* 'Bua Phuean' has been rapidly decreased owing to the growth of urbanization and industrialization and other land use that caused 'Bua Phuean' habitat erosion. Though this plant was generally propagated via rhizome, multiplication from seed could efficiently used to preserve the genetic variability (Sumlu et al., 2010). In this study, 'Bua Phuean' was successfully multiplied through seed germination in vitro which could assist to escalate the quantity and lessen the threat of this plant in natural habitat. Seed germination of 'Bua Phuean' in our experiment did not require cold stratification or perform any dormancy like in some reports (Estrelles et al., 2004; Sumlu et al., 2010). However, seed maturity would possibly play the key role in 'Bua Phuean' seed germination as the fastest and highest germination rate was found in stage 5.

In the present study, seeds from stage 4 and 5 apparently had the potential for plant propagation even though the highest survival rate was from stage 5. Since 'Bua Phuean' was observed in many regions of Thailand, it is worth to compare the duration of seed development at each stage, particularly stage 4 and 5, under different environments and germination rate from our techniques. This will be useful for rapid seed stage selection and reduce the time for propagation at the proper stage and condition. However, using different age of seeds in stage 4 and 5 to reproduce has many points to be continuously investigated and compared such as, growth, reproductivity, environmental tolerance and disease resistance because these examinations could make the completion on the development of this alternative technology in the future.

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